Nitrogen Balance of Women Consuming Cottonseed Protein

B.B. ALFORD, S. KIM, and K. ONLEY, Department of Nutrition and Food Sciences, Texas Woman's University, Denton, Texas 76204

ABSTRACT AND SUMMARY

This study investigated the ability of cottonseed protein in liquid formula diets to maintain the nitrogen balance of twelve young women at the level of protein intake usually eaten by adults in the United States. Four levels of defatted cottonseed flour (0, 30, 60, and 100%) and R1-5, a commercially available protein product presumed to be adequate to support nitrogen balance, were used to provide 10 grams of nitrogen. After equilibration each diet was fed for 7 days. Mean daily nitrogen balances were -0.05, S.D. 1.28, +0.76, S.D. 1.62; +0.35, S.D. 2.46; and +1.42, S.D. 1.88 for the 0, 30, 60, and 100% cottonseed diets. The ratios of essential to nonessential amino acids were 0.82, 0.76, 0.67, and 0.59 for the diets from 0 to 100% cottonseed. At the level fed in this study, cottonseed protein adequately maintained nitrogen equilibrium in young adult women.

INTRODUCTION

Oilseed proteins play an important role in supplying the protein needs of an ever-increasing world population. Since the development by Vix et al. (1) of the liquid cyclone process (LCP) for the removal of the pigment gossypol from glanded cottonseed protein, this food source has become a possibility for meeting human protein needs. Cottonseed protein has been used in South America in limited amounts as part of INCAPARINA #9 (2). Graham et al. (3,4) reported successful use of cottonseed protein to rehabilitate malnourished infants. Srikantia and Shagal (5) in India demonstrated low gossypol cottonseed protein was useful for malnourished children. There is a need to study nitrogen balance of adults consuming cottonseed protein to determine the nutritional quality.

The purpose of this study was to investigate the ability of cottonseed protein in the diets to maintain nitrogen balance of young women. The levels of protein were representative of the many American dietary patterns.

EXPERIMENTAL PROCEDURES

Twelve young university women were maintained on liquid formula diets for a period of 5 wk. The study period was completed before final examination schedules. Overall protein status was determined by assessing nitrogen balance of subjects fed varying levels of cottonseed protein.

Subjects

The twelve young college women, aged 19 to 24 yr, were of normal weight and in good health as determined by medical history and examination. The weight of the subjects ranged from 52.2 to 86.8 kg with a mean of 60.7 kg. For three weeks prior to the study each subject maintained complete food intake records. Subjects were weighed twice weekly during this period. Caloric needs were determined from the actual caloric intake needed to maintain a stable weight and by measurement of basal metabolism. For the 35 day experiment, subjects continued daily activities and were housed together in one section of a dormitory.

Diets

Since energy and protein requirements are related, each diet provided the caloric level required by the individual subject. The range was 1900 to 2250 calories. The diets were designed to provide 13% of the total calories from protein. This resulted in a range of 9.9 to 11.7 g of nitrogen provided.

Cottonseed protein was compared to a commercially available protein preparation, R1-5, presumed to be adequate to support nitrogen balance. The defatted glanded cottonseed flour, Liquid Cyclone Process (LCP), used in this study was produced by the Southern Marketing and Nutrition Research Division, USDA (New Orleans, LA). The reference protein, R1-5, was obtained from Ross Laboratories (Columbus, OH). R1-5 was developed for use in metabolic studies. Ahrens (6) reports satisfaction with R1-5. Liquid formula diets were prepared using four different levels of cottonseed flour providing 0, 30, 60, and 100% of the protein, with the remainder of the protein being supplied by R1-5. The proteins were blended with corn oil, dextrose, salt, and water. The composition of the diets is shown in Tables I and II. Formula diets were made daily and an aliquot of each was frozen.

The aliquot of individuals' diets was analyzed for nitrogen. Variations in actual nitrogen content were found. All diets ingredients were carefully measured. The protein tended to cling to the blender walls. Although every attempt was made to remove this, some nitrogen may have been lost by this means. The liquid formula diet was given under supervision as four equal meals daily at ca. 8:00 A.M., 12:00 P.M., 3:00 P.M., and 7:00 P.M. Each subject consumed all of the diet.

Period of Study

The 35-day study period was divided as follows. For the first 7 days all subjects consumed a 100 per cent R1-5 diet to allow for adjustment to a liquid formula regime. Following the adjustment period the four experimental diets were administered for 7 days each in a randomized pattern with the subjects divided into four groups, each group receiving a different diet. Throughout the study total dietary nitrogen was relatively constant for each subject. Of each 7 day period only the data of the last 4 days was used for determination of nitrogen balance.

Nitrogen Balance

The gross nitrogen balance was determined by deducting fecal, urinary, and integumental nitrogen from dietary nitrogen intake for each subject and day. For integumental loss a value of 150 mg of nitorgen daily was used. This adjustment for integumentary nitrogen loss per day was based on the study of Sirbu et al. (7) and reported menstrual losses (8). The value allows 25 mg for hair and nail growth, 125 mg for insensible perspiration and menstrual losses. Insensible perspiration results in 100 mg N loss daily at a 7 g nitrogen intake (7). Menstrual losses vary from 11 to 29 mg nitrogen per day over the 28 day cycle based on menstrual period losses between 300 to 800 mg nitrogen (8). A mean of the 4 day fecal nitrogen excretion was used in the daily calculation. Statistical analysis utilized value of the grams of nitrogen balance per day, per individual.

Sample Collection and Handling

Complete 24-hr urine collections were made throughout the experiment, measured, and an aliquot was taken from the individual 24-hr collections and frozen. Brilliant blue,

TABLE I

Mean Composition of Experimental Formula Diets[a]

Dietary protein source	Liquid cyclone process g/day	R1-5 g/day	Corn oil g/day	Dextrose g/day	NaCl	Water ml/day
100% R1-5	0	129.5 ± 6.5	78.8 ± 4.0	174.5 ± 10.5	2.0	820
70% R1-5 30% LCP	31.4 ± 2.5	90.7 ± 1.0	76.5 ± 5.3	180.0 ± 15.5	2.0	820
40% R1-5 60% LCP	72.4 ± 4.5	54.8 ± 5.5	77.5 ± 4.5	186.0 ± 16.3	2.0	820
100% LCP	104.8 ± 5.5	0	78.5 ± 4.5	195.5 ± 16.3	2.0	820
Glycerolmonooleate (1.0 gm each) used as an emulsifying agent						

Addition to liquid formula diet:

Apples, celery	
Lemon, salt, low calorie soft drink	Variable
Methylcellulose wafers	Allowed up to 36 Cal.
Vitamin supplements[b]	
Mineral supplements[c]	

[a]Ranges according to body weight of individual subjects.

[b]Unicaps-M (Upjohn). Each pill contains: vitamin A 5,000 USP units, vitamin D 400 USP units, riboflavin 2.5 mg, thiamin mononitrate 2.5 mg, ascorbic acid 50 mg, niacinamide 20 mg, vitamin B_6 0.5 mg, B_{12} 2 μg, calcium pantothenate 5 mg, vitamin E 10 IU. Folic acid 1 mg every other day.

[c]Total mineral supplements were in milligrams: Iron 10, iodine 0.15, copper 1.0, manganese 1.0, magnesium 6, potassium 5.0, calcium 800.

TABLE II

Selected Amino Acid Composition of Experimental Formula Diets

Amino acids in diets (g/day)	100% RI-5	30% LCP[b] 70% RI-5	60% LCP 40% RI-5	100% LCP
Essential amino acids (EAA)				
lysine	3.48	3.09	2.96	2.34
threonine	3.03	2.61	2.50	2.38
valine	4.91	4.90	4.23	3.61
meth + cyst	4.06	3.40	3.13	2.86
isoleucine	3.86	3.76	3.68	3.02
leucine	5.25	5.07	3.98	3.21
phenylalanine	2.63	2.63	2.41	2.92
tryptophan	0.71	0.72	0.70	0.69
Total	27.93	26.18	23.59	21.03
Nonessential amino acids (NEAA)				
histidine	1.21	1.24	1.33	1.43
arginine	1.95	3.11	4.12	5.53
aspartic acid	3.99	4.28	4.65	4.91
serine	3.78	3.70	3.42	3.42
glutamic acid	12.22	11.72	11.32	11.24
proline	5.37	4.39	3.76	2.42
glycine	1.06	1.71	2.13	2.24
alanine	1.91	1.93	1.99	2.16
tyrosine	2.48	2.30	2.26	2.24
Total	33.97	34.38	34.98	35.59

[a]Each is the mean value of three separate hydrolysate preparations.

[b]LCP = liquid cyclone process.

FCF, capsules were given to the subjects as fecal markers to indicate the beginning of the four day period used for balance data and carmine markers on the last day of each seven day period. The fecal samples were frozen after delivery to the laboratory. Fecal samples were pooled for the 4 day period, blended in a blender with deionized water, and aliquots were weighed for nitrogen assays.

Analyses of Samples

Dietary nitrogen and urinary nitrogen excretion were determined daily although only the final 4 days of each 7 day period were used for balance determinations. The mean daily fecal nitrogen output was determined from pooled 4 day samples. Because of the homogeneous nature of the sample, urinary nitrogen was determined by the conventional micro-Kjeldahl technique with slight modification as suggested by Henry (9), replacing mercuric oxide with cupric sulfate as the digestion catalyst. In order to utilize a larger sample, dietary and fecal nitrogens were determined by the macro-Kjeldahl procedure. Determination of creatinine in the urine was done by the Biggs and Cooper (10) modification of the Folin-Wu procedure.

The amino acids in LCP, R1-5, and each of the formula diets were analyzed by ion exchange chromatography on a Beckman 121 M microcolumn automatic amino acid analyzer (11). Hydrolysis of samples was performed with 6 N HCl at 110 C for 24 hr in a forced-draft oven to obtain hydrolysates suitable for analysis of all amino acids except cystine plus cysteine and tryptophan. After hydrolysis, the HCl was removed from the hydrolysate mixture by rotary evaporation. To correct for the destruction of serine, threonine, and the branched chain amino acids, three hydrolysis times (24, 48, and 72 hr) were used and extrapolated to zero and infinite time according to a

TABLE III

Nitrogen Balance of Twelve Young Women on Diets with Varying Percentages of Cottonseed Protein

Dietary protein source	Nitrogen intake[a,b] (g/day)		Urinary excretion[a,b] (g/day)		Fecal excretion[b] (g/day)		Nitrogen balance[b] (g/day)		Creatinine excretion (g/day)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
100% RI-5	10.13	(0.95)	9.59	(2.03)	0.59	(0.25)	-0.05	(2.18)	0.79	(0.12)
Range	11.97- 9.03		6.93- 14.03		.18- 1.01		-4.38 to +2.30		.53- .98	
70% RI-5 30% LCP[d]	9.95	(0.73)	8.58	(1.91)	0.61	(0.18)	+0.76	(1.62)	0.72	(0.20)
Range	8.79- 11.00		5.45- 10.48		.44- 1.07		-1.97 to +2.98		0.47- 0.99	
40% RI-5 60% LCP	10.41	(0.57)	9.38	(2.48)	0.68	(0.21)	+0.35	(2.46)	0.75	(0.18)
Range	9.00- 11.23		5.32- 14.02		.41- 1.13		-4.67 to +5.14		0.45- 0.97	
100% LCP	10.14	(0.70)	7.98	(1.35)	0.74	(0.25)	+1.42	(1.88)	0.77	(0.16)
Range	9.00- 11.4		6.71- 10.20		.49- 1.23		-1.83 to 3.58		0.57- 1.16	

[a]In grams per day based on nitrogen intakes of 9.95-10.41 (13% of the total calories as protein).

[b]Values (g N/day) based on final 4 days of the 7 day dietary periods.

[c]Total integumental loss such as skin, hair, nail, sweat was counted as 0.15 g/day and was computed in urinary excretion.

[d]LCP = liquid cyclone process.

procedure by Tkachuk (12).

For the measurement of cystine plus cysteine as cysteic acid and of methionine as the sulfone, a sample was oxidized with performic acid by the modified procedure of Moore (13). For tryptophan, samples were subjected to alkaline [$Ba(OH)_2 \cdot H_2O$] hydrolysis and measured colorimetrically using p-dimenthylamino-benzaldehyde according to the method described by Miller (14).

RESULTS AND DISCUSSION

Nitrogen Balance

The mean nitrogen balances of subjects receiving the four different diets during the final 4 days of the 7 day experimental periods are shown in Table III. One way analyses of variance with repeated measures, indicated mean differences between diets at smaller than the 5% level of significance. All values are within the equilibration zone by definition of retention of ±5 per cent of nitrogen intake (15-18).

Most of the subjects maintained positive nitrogen balance with a mean nitrogen intake of 10.16 g per day with the calculations including an allowance for integumental losses. Although 100% R1-5 diet resulted in a slightly negative balance with a loss of 0.05 g nitrogen, it would still be considered within the equilibration range. All diets containing LCP produced slightly positive nitrogen balances with the 100% LCP diet giving the most positive results.

The better balances on all cottonseed protein are difficult to explain. The high level of nitrogen used in the study is a factor. Steffee et al. (19) have indicated that molecular nitrogen may be an important factor in nitrogen loss at generous intakes of protein in healthy subjects. These losses may vary with the source of protein fed and are not measured in the balance technique. Although nitrogen as ammonia is limited in intestinal gas no measure of this possible source of nitrogen loss was made. When consuming the all cottonseed protein diet more subjects reported mild symptoms of intestinal gas noteably remarking about fullness and a mildly distended abdomen. Unmeasured nitrogen losses result in a higher apparent balance.

It has been observed that creatinine excretion for adults is related to the dietary protein intake (20,21). Overall

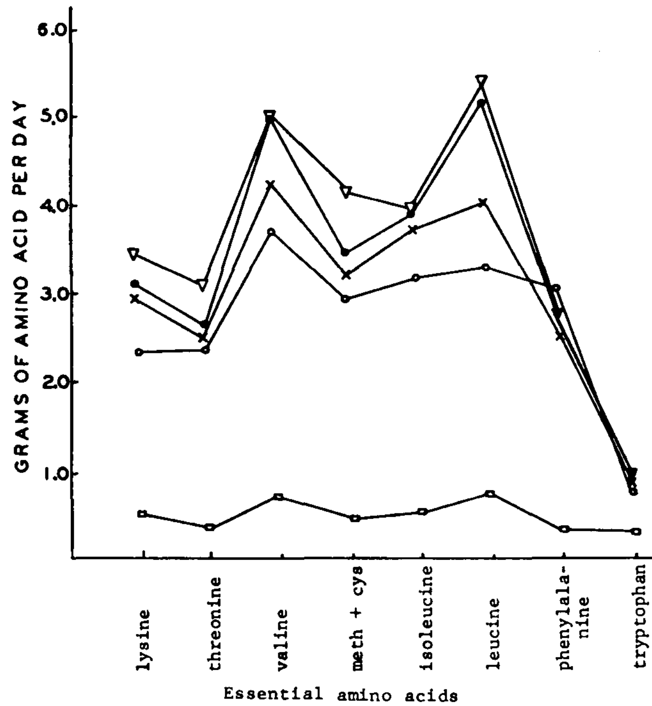

FIG. 1. Essential amino acids. Relationship between minimum daily requirement of essential amino acids for young women and amounts of amino acids supplied by study diets. □ requirement, X 60% liquid cyclone process (LCP) 40% rR1-5, ▽ 100% R1-5 diet, ○ 100% LCP, ● 30% LCP, 70% R1-5.

creatinine excretion was ca. one-half of the values reported when meat diets are fed (22).

Amino Acid Composition of the Diet

The major differences in essential amino acid content between the diets were in the greater content of lysine, methionine, leucine, and valine in R1-5. Among the nonessential amino acids differences occurred in arginine and glycine with the cottonseed diet containing more, while more proline was found in the R1-5 diet. The proportion of essential to nonessential amino acids in cottonseed protein is lower when compared to an animal reference protein.

TABLE IV

Nitrogen Balance Status of Twelve Subjects for all Experimental Periods

	Experimental period							
	1		2		3		4	
Case number	Diet	N balance[a]	Diet	N balance	Diet	N balance	Diet	N balance
2977	B	-0.88	C	+1.56	A	+2.30	D	+3.58
2978	D	+0.10	A	-1.10	B	-1.97	C	-1.01
2979	C	+5.14	B	+2.66	A	+2.98	D	+3.21
2980	C	-1.03	D	+1.19	A	-4.38	B	-0.66
2981	B	+2.90	C	+1.95	D	+0.34	A	-0.75
2982	B	+0.22	D	+1.09	C	-4.67	A	-3.23
2983	D	-1.83	A	-0.01	B	+2.12	C	-1.81
2984	A	-0.82	D	+2.69	C	+2.28	B	-0.03
2985	A	-0.52	C	-2.23	D	+0.25	B	+0.12
2986	C	-0.25	B	-0.21	D	+1.13	A	+0.94
2987	D	+1.43	A	+1.70	B	+0.76	C	-0.39
2988	A	+2.16	B	+2.98	C	+4.53	D	+3.76

[a]A = 100% RI-5; B = 30% liquid cyclone process (LCP), 70% RI-5; C = 60% LCP, 40% RI-5; D = 100% LCP.

From the values in Table II, the daily intake of essential amino acids relative to the total nitrogen decreases as the proportion of cottonseed flour increases. Although the total essential amino acids supplied by diet containing only cottonseed protein is slightly lower than the other diets, this amount is above reported adult requirements (23).

All diets contained at least three times the minimal requirements of crystalline essential amino acids for women (24) (Fig. 1). Moreover, total sulfur-containing amino acids were considerably higher than the amount of methionine required in the absence of cystine.

The variability in the nitrogen balance among some of the subjects (see TAble IV) could be due to the variation in the utilization of amino acids (25). In addition, individual factors such as emotional stress or depression effect balance. These women were students and maintaining regular activities; and thus were subject to normal life stresses.

There appears to be little difference in the digestibility of the two proteins fed to the subjects, with both proteins very digestible. Values for fecal nitrogen were similar for all diets and were relatively low. The digestibility for the proteins was 92.7% and 94.2% for diets with 100% cottonseed and R1-5, respectively.

At the high level of intake usually consumed by populations in the United States, cottonseed protein alone or in combination with animal protein maintains nitrogen balance. Further work needs to be conducted to determine the minimum nitrogen required to maintain balance when the protein source is cottonseed to provide a critical measure of the quality of cottonseed protein. The 13% level in this study only provided preliminary information, for low quality proteins maintain balance in adults when fed in generous amounts.

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